Rec'd PCT/PTO SEP 2004
WO 03/079029 PCT/US03/07724
10/506930

# POSITIONING SYSTEM FOR MOVING A SELECTED STATION OF A HOLDING PLATE TO A PREDETERMINED LOCATION FOR INTERACTION WITH A PROBE

The present application is a continuation-in-part of pending U.S. Patent Application Serial No. 09/894,956 filed June 27, 2001, which is a continuation-in-part of pending U.S. Patent Application Serial No. 09/687,219, filed October 12, 2000, which is a continuation-in-part of pending U.S. Patent Application Serial No. 09/444,112, filed November 22, 1999, which is a continuation-in-part of pending U.S. Patent Application Serial No. 08/876,276, filed June 16, 1997; additionally, the present application is a continuation-in-part of pending U.S. Patent Application Serial No. 09/636,778, filed August 11, 2000, which application is a continuation and claims the benefit of priority under 35 U.S.C. § 120 of U.S. Patent Application Serial No. 09/098,206, filed June 16, 1998, which issued as U.S. Patent No. 6,174,673 on January 16, 2001, which is a continuation-in-part of pending U. S. Patent Application Serial No. 08/876,276, filed June 16, 1997, all of the contents of which are incorporated by reference in their entirety herein.

## FIELD OF THE INVENTION

The present invention pertains generally to devices for performing operations on selected samples in a holding plate with a probe. More particularly, the present invention pertains to positioning systems for moving a selected station of a holding plate to a predetermined location for interaction with a probe. The present invention is particularly, but not exclusively, useful as a computer assisted, optical system for positioning a holding plate having over a thousand, small diameter through-hole stations at a precise location to allow a probe to interact with a selected station.

## BACKGROUND OF THE INVENTION

Plates for holding specimen samples in a fluid solution are available having over a thousand, small diameter stations. The stations can include through-holes, or wells that extend only partially into the holding plate. In the case of a through-hole station, these stations rely on surface tension to hold each fluid sample in a respective station. The through-hole stations of a holding plate can be filled with a solution of interest by simply immersing a surface of the holding plate into the solution. Capillary action causes the solution to enter the through-hole stations. This allows a very large number of relatively small volume samples of the solution to be simultaneously prepared for later analysis or manipulation. Specifically, holding plates having over a thousand stations arranged in a planar array, with station diameters of only about 500 microns, are available.

Once the holding plate has been filled with solution, it is often desirable to either add material to selected stations or withdraw solution from selected stations. This is particularly the case when the solution used to fill the holding plate is non-homogenous. Often times, the selected stations differ in color, opacity, fluorescence or are otherwise optically distinguishable from the remaining stations. For example, a biological or chemical reaction may proceed more rapidly in portions of the solution, causing only selected stations to change color, while the remaining stations do not. Withdrawal of solution from the selected stations allows for the separation of the solution into portions of solution that have reacted and portions of solution that have not reacted. Alternatively, it may be desirable to add a material such as a chemical reagent to selected stations, again selecting stations based on some optical property of the sample in the station.

Generally, a thin, needle-like probe must be positioned in fluid communication with a selected station to either add or withdraw material from the station. Thus, it is often desirable to select a specific station based on an optical characteristic of the station's sample and then operate on the selected station with a probe. To accomplish this, the probe and selected station must first be aligned. Unfortunately, for stations having extremely small diameters, such as through-

holes with diameters of 500 microns or less, it is impossible for all practical purposes, to manually align a selected station with a probe. Thus, the present invention recognizes that a computer-assisted, automated system is necessary to align small diameter stations with a probe.

Holding plates are generally designed with stations (i.e. through-holes or wells) having station axes that are perpendicular to the sides of the holding plate. With this design, the axes of the stations are relatively easily aligned with the path of the probe. Unfortunately, due to defects in the manufacturing processes that are used to prepare the holding plates, the axes of the stations can sometimes be misaligned, albeit slightly, from the sides of the holding plate. Stated another way, an end of the station on one side of a holding plate is offset from the other end of the station on the opposite side of the holding plate. It is to be appreciated that this offset can present problems when imaging is performed on one side of the holding plate while the probe is aligned with the station on the opposite side of the holding plate. The problem becomes more egregious with respective increases in the aspect ratio of the station, the density of stations on the plate and the thickness of the plate.

It is often the case that hundreds of stations (among the thousand or more stations present in the holding plate) may require interaction with the probe. In these cases, it becomes too labor intensive for an operator to select each station individually for interaction with the probe. Thus, it would be desirable to have a computer-assisted system that allows the operator to select a set of stations by merely choosing an optical characteristic to establish the set. With the set established, the operator then instructs the computer to successively perform a probe operation on each station in the selected set. A convenient system would allow an operator to specify an optical characteristic, for example - fluorescence, and then instruct the computer to make a chemical addition to each station having a green sample that is fluorescing.

In light of the above, it is an object of the present invention to provide a system suitable for the purposes of moving a selected station of a holding plate to a predetermined location for interaction with a probe. It is another object of the present invention to provide a positioning system for aligning a probe and selected station wherein the station has an extremely small diameter (i.e. a through-hole

having a diameter of 500 microns or less). It is yet another object of the present invention to provide a system for automatically performing a probe operation on samples in a selected set of stations that all have a common optical characteristic. Still another object of the present invention is to provide a positioning system for aligning a probe with a selected station wherein the station axis is offset (i.e. at a non-normal angle) relative to the side of the holding plate. Yet another object of the present invention is to provide an optical positioning system for aligning a small diameter through-hole station with a probe which is efficient to use, relatively simple to implement, and comparatively cost effective.

## SUMMARY OF THE INVENTION

The present invention is directed to a device for positioning the tip of a probe at a selected station of a holding plate. For the present invention, the holding plate is formed with a substantially flat first side and an opposed second side. Preferably, the holding plate is further formed with a regular or irregular planar array of stations for holding a plurality of respective samples. Importantly, each station is accessible by the probe from the first side of the holding plate.

In accordance with the present invention, the probe is attached to a base and a mechanism is provided to allow for reciprocal movement of the probe relative to the base. The device further includes a moveable stage that is mounted on the base to support the holding plate. For the present invention, the moveable stage is formed with a planar surface for engagement with the second side of the holding plate. With this cooperation of structure, the planar surface of the stage defines a coordinate plane ($m_{xy}$) containing orthogonal axes x and y. A mechanism is provided to secure the holding plate to the stage, causing the holding plate to move with the stage. With the second side of the holding plate secured against the stage, the first side of the holding plate remains exposed for interaction with the probe. To selectively move the stage (and the holding plate) in the x and y directions relative to the base and probe, the device further includes a pair of motorized linear actuators.

As indicated above, the probe is attached to the base. In greater structural detail, the probe is elongated and defines a probe axis in the direction of elongation. For the present invention, the elongated probe is optically distinguishable and, for this purpose, is preferably mounted on a fluorescent hub and extends from the fluorescent hub to a probe tip. The hub, in turn, is mounted on the base. Importantly for the present invention, the probe is positioned relative to the holding plate to allow the tip of the probe to interact with the first side of the holding plate. Additionally, the probe and hub are preferably mounted on the base with the probe axis of the probe oriented normal to the $m_{xy}$ plane. In the preferred embodiment of the present invention, a mechanism is provided to allow the probe to reciprocate (relative to the holding plate and base) along the probe axis and in a direction that is substantially orthogonal to the $m_{xy}$ plane. With the above described combination of structure, the motorized linear actuators can be used to move the holding plate to a location in the $m_{xy}$ plane such that a selected station is positioned on the probe axis. With the selected station positioned on the probe axis, the probe can be moved along the probe axis to interact with the selected station.

To locate a selected station of the holding plate at a position on the probe axis, the device includes at least one camera and a computer processor. In the preferred embodiment of the present invention, the camera is positioned on the probe axis and oriented to obtain a pixel image of the holding plate stations from the second side of the holding plate. To facilitate imaging from the second side of the holding plate, a transparent stage is preferably used. Alternatively, one or more holes can be formed in the stage to allow the camera to image the stations from the second side of the holding plate.

In operation, the device is initially calibrated (calibration procedure described below). Next, a first holding plate is installed on the stage, placing the holding plate at a first location in the $m_{xy}$ plane. The expectation at this point is that there will be an optical contrast between various stations in the holding plate. One or more pixel images are then obtained by the camera that images the array of stations positioned at the first location in the $m_{xy}$ plane and the projection of the probe in the $m_{xy}$ plane. For the present invention, the pixel image defines a

coordinate plane ($p_{xy}$) that is related to the coordinate plane ($m_{xy}$). From the pixel image, the operator selects a specific station of the holding plate that requires interaction with the probe. This information is then transferred to the computer processor. The computer processor instructs the motorized linear actuators to move the holding plate through the proper x and y distances in the $m_{xy}$ plane to align the selected station on the probe axis. More specifically, the computer uses a relationship that was previously established between the coordinate plane ($p_{xy}$) and the coordinate plane ($m_{xy}$) during calibration to accurately move the stage and align the selected station on the probe axis. With the selected station positioned on the probe axis, the probe is then translated along the probe axis to interact with the station. In one embodiment of the present invention, station offset information (i.e. the deviation of each station axis from a reference axis that is orthogonal to the side of the holding plate) is input into the computer processor. The computer processor then uses the offset information to ensure that the station entrance located at the first side of the holding plate is aligned with the probe axis.

To calibrate the device, an optical marker is placed on the stage and a first pixel image is obtained by the camera. As such, the first pixel image includes the optical marker positioned at a first location in the $m_{xy}$ plane. Preferably, the calibration procedure is performed without a holding plate on the stage. Next, the stage is moved using the motorized linear actuators to successive locations in the $m_{xy}$ plane. The actuator displacements (e.g. motor steps) necessary to move the optical marker between locations are recorded and a pixel image of the optical marker is obtained at each location. These pixel images and actuator displacements are then used by the computer processor to correspond the $p_{xy}$ coordinate plane with the $m_{xy}$ coordinate plane. Stated another way, the pixel images are used to find the relationship between the $p_{xy}$ coordinate plane and the $m_{xy}$ coordinate plane. Preferably, the method of least squares is used to establish an approximate linear relationship between the coordinate plane ($p_{xy}$) and the coordinate plane ($m_{xy}$).

## BRIEF DESCRIPTION OF THE DRAWINGS

The novel features of this invention, as well as the invention itself, both as to its structure and its operation, will be best understood from the accompanying drawings, taken in conjunction with the accompanying description, in which similar reference characters refer to similar parts, and in which:

Fig. 1 is a perspective view of a device in accordance with the present invention for moving a selected station of a holding plate to a predetermined location for interaction with a probe;

Fig. 2 is an enlarged, sectional view of a portion of a holding plate and stage as would be seen along line 2-2 in Fig. 1;

Fig. 3A is an exemplary pixel image taken after the optical marker has been moved to a first location;

Fig. 3B is an exemplary pixel image taken after the optical marker has been moved to a second location;

Fig. 3C is an exemplary pixel image taken after the optical marker has been moved to a third location; and

Fig. 4 is a sectional view as in Fig. 2 showing a holding plate with offset stations.

## DESCRIPTION OF THE PREFERRED EMBODIMENT

Referring initially to Fig. 1, a system 10 for performing operations on selected samples in a holding plate 12 with a probe 14 in accordance with the present invention is shown. As shown, the system 10 includes a base 16 for supporting both the holding plate 12 and the probe 14. As further shown, the probe 14 is preferably elongated and defines a probe axis 18 in the direction of elongation. In the preferred embodiment of the present invention, the probe 14 is formed as a hollow needle having a lumen capable of transferring fluid. Also shown in Fig. 1, the elongated probe 14 is preferably mounted on a hub 20 and extends from the hub 20 to a probe tip 22. For the present invention, the hub 20, which is preferably fluorescent, is somehow optically distinguishable from the probe 14. The system 10 also includes a mechanism 24 to move the probe 14 back and forth along the probe axis 18, relative to the base 16 and holding plate 12. Those skilled in the art will appreciate that any mechanism 24 known in the pertinent art for reciprocating a probe back and forth along an axis, such as a hydraulic or pneumatic cylinder, can be used in conjunction with the present invention.

With cross reference now to Figs. 1 and 2, it can be seen that the holding plate 12 is formed with a substantially flat first side 26 and an opposed second side 28. Preferably, the holding plate 12 is further formed with a regular or irregular planar array of stations 30, for which stations 30a-c shown in Fig. 2 are exemplary. Each station 30 is provided to hold a fluid sample and may optionally be a through-hole that extends through the plate 12 between sides 26 and 28. In the preferred embodiment of the present invention, the holding plate 12 is formed with over one thousand stations 30, with each station 30 having an inner diameter 31 of approximately 500 microns or less. An optional coating 32 can be applied to each through-hole station 30 to limit the transmission of light between adjacent stations 30. Importantly, each station 30 is accessible by the probe 14 from the first side 26 of the holding plate 12.

With continued cross reference to Figs. 1 and 2, it can be seen that the system 10 further includes a moveable stage 34 that is mounted on the base 16 to support the holding plate 12. As further shown, the moveable stage 34 is formed with a planar surface 36 for engagement with the second side 28 of the holding plate 12. As shown, the planar surface 36 of the stage 34 defines a coordinate plane ($m_{xy}$) containing orthogonal axes x and y. If required, clamps (not shown) can be provided to secure the holding plate 12 to the stage 34. In any case, with the holding plate 12 on the stage 34, the stage 34 and holding plate 12 move together. With the second side 28 of the holding plate 12 secured against the stage 34, the first side 26 of the holding plate 12 remains exposed for interaction with the probe 14.

As best seen in Fig. 1, the system 10 includes a pair of motorized linear actuators 38a, b that are mounted on the base 16 to selectively move the stage 34 and holding plate 12 in the x and y directions relative to the base 16 and probe 14. It is to be further appreciated that the motorized linear actuators 38a, b move the holding plate 12 within the $m_{xy}$ plane. Preferably, each motorized linear actuator 38a, b includes a stepper motor for driving a lead screw to move the stage 34. For the present invention, any type or number of motorized linear actuators or other devices known in the pertinent art for selectively moving a stage in at least two directions can be used.

Referring now with cross reference to Figs. 1 and 2, it can be seen that the probe 14 is positioned relative to the holding plate 12 to allow the probe tip 22 to interact with the first side 26 of the holding plate 12. Additionally, the probe 14 is preferably mounted on the base 16 with the probe axis 18 of the probe 14 oriented normal to the $m_{xy}$ plane (i.e. the plane containing the x and y axes). Thus, the probe 14 reciprocates along the probe axis 18 and in a direction that is orthogonal to the $m_{xy}$ plane. In accordance with the present invention, the motorized linear actuators 38a, b can be selectively activated to move the holding plate 12 to a location in the $m_{xy}$ plane such that a selected station 30 is positioned on the probe axis 18. With the selected station 30 positioned on the probe axis 18, the probe 14 can then be moved along the probe axis 18 to interact with the selected station 30. More specifically, the probe 14 can manipulate a sample that is held by the

holding plate 12 at the selected station 30. Manipulations of the sample by the probe 14 can include sample withdrawal from the station 30 or the addition of a material such as a chemical reagent to the sample.

As best seen in Fig. 1, the system 10 includes a camera 40 and a computer processor 42 with a display 44. Preferably, as shown, the camera 40 is positioned on the probe axis 18 and oriented to image the stations 30 of the holding plate 12 from the second side 28 (shown in Fig. 2) of the holding plate 12. The camera 40 produces a pixel image 46 that can be displayed on the display 44. The holding plate 12 can be imaged through transparent portions of the stage 34 and base 16, or one or more holes can be formed in the stage 34 and base 16.

In the preferred embodiment of the present invention, the system 10 further includes an illumination system 48 for illuminating and / or exciting samples in the holding plate 12. For example, the illumination system 48 can be used to excite fluorescent materials in the holding plate 12. In accordance with the present invention, one or more light filters 50 can be used to selectively filter light entering the camera 40. For example, light filter 50 can be used to filter out backscattered excitation light from illumination system 48 while allowing fluorescent emissions from the samples to be imaged by the camera 40.

In operation, a holding plate 12 is installed on the stage 34, as shown in Fig. 1 and a pixel image 46 is created by camera 40 and presented in a viewable format by display 44. As shown, the pixel image 46 sequentially includes a hub image 52, a probe image 54 and an image of the array of stations 30 of the holding plate 12. In part, because the probe 14 is surrounded by an optically distinguishable hub 20, the relatively thin probe 14 can be imaged. It is to be appreciated that the pixel image 46 also shows stations 30, including stations 30 that have distinguishing optical characteristics (e.g. color, fluorescence, opacity, etc). In Fig. 1, pixel image 46 shows the image of five selected stations 30 that have distinguishing optical characteristics (i.e. selected stations image 56).

As indicated above, the function of the system 10 is to move the holding plate 12 within the $m_{xy}$ plane to position a selected station 30 on the probe axis 18. With the selected station 30 on the probe axis 18, the probe 14 is then moved

along the probe axis 18 to manipulate a sample in the selected station 30. For the present invention, the pixel image 46 defines a coordinate plane ($p_{xy}$) that is related to the coordinate plane ($m_{xy}$). In accordance with the present invention, stations 30 are selected in the pixel image 46 for manipulation by the probe 14. The computer processor 42 then instructs the motorized linear actuators 38a, b to move the holding plate 12 within the $m_{xy}$ plane to position the selected station 30 on the probe axis 18. In accordance with the present invention, the system 10 is calibrated to accomplish this movement with extremely small positional errors. During calibration, the computer processor 42 determines the relationship (i.e. correspondence) between the coordinate plane ($p_{xy}$) and the coordinate plane ($m_{xy}$).

To establish the relationship between the coordinate plane ($p_{xy}$) and the coordinate plane ($m_{xy}$), an optical marker is placed on the stage 34 and the stage 34 is moved via the motorized linear actuators 38a, b to successive locations in the $m_{xy}$ plane. A separate pixel image 46 is obtained at each location. The displacements of the motorized linear actuators 38a, b (e.g. motor steps) necessary to move the optical marker from the first location to the second location and from the second location to the third location are recorded and input into the processor 42.

Figs. 3A, 3B and 3C show pixel images 46', 46" and 46'" for three locations of the stage 34 within the $m_{xy}$ plane. In greater detail, Fig. 3A shows pixel image 46' for stage 34 in a first location and includes an optical marker image 58'. Similarly, Fig. 3B shows pixel image 46" for stage 34 in a second location and includes an optical marker image 58". Also, Fig. 3C shows pixel image 46'" for stage 34 in a third location and includes an optical marker image 58'". Although pixel images 46', 46" and 46'" for three stage 34 locations are shown herein, it is to be appreciated that any number of locations can be used with the present invention to establish a relationship between the coordinate plane ($p_{xy}$) and the coordinate plane ($m_{xy}$). Once the displacements of the motorized linear actuators 38a, b (e.g. motor steps) and pixel images 46', 46" and 46'" have been obtained, a linear regression technique, such as the method of least squares, can be used

by the processor 42 to establish an approximate linear relationship between the coordinate plane ($p_{xy}$) and the coordinate plane ($m_{xy}$) to calibrate the system 10.

Referring now to Fig. 4, a portion of a holding plate 12 having a thickness, "t", is shown. The holding plate 12 includes a station 30 with a station entrance (top) 60 that is offset from the station exit (bottom) 62. As further shown, the axis 64 of the station 30 is inclined at an angle, $\alpha$, from an axis 66. More specifically, the axis 66 is normal to the side 26 of the holding plate 12 and passes through the exit (bottom) 62. It can be further seen that a line 67 on side 26, which intersects both the axis 66 and the axis 64 establishes a rotation angle, $\theta$, between the line 67 and a base reference line 68 about the axis 66. In one embodiment of the present invention, this offset information (i.e. $\alpha$, $\theta$, and "t") for the plate 12 is input into the computer processor 42. With this offset information, the computer processor 42 uses an image of the second side 28 of the plate 12 to accurately locate the entrance 60 of the plate 12 on the probe axis 18 (probe axis 18 shown in Fig. 1).

While the particular positioning system for moving a selected station of a holding plate to a predetermined location for interaction with a probe as herein shown and disclosed in detail is fully capable of obtaining the objects and providing the advantages herein before stated, it is to be understood that it is merely illustrative of the presently preferred embodiments of the invention and that no limitations are intended to the details of construction or design herein shown other than as described in the appended claims.